Remarks

By way of the foregoing amendment, Claims 29, 30, 35 and 44-59, 63, 65, 67, 69, and 71-72 are currently pending. Claims 30, 57 and 62 have been withdrawn by the Examiner as being drawn to nonelected inventions. Claims 1-28, 31-34 and 36-43 have been previously cancelled, and Claims 60-62, 64, 66, 68, 70, and 73 are hereby cancelled. Claim 69 has been amended without prejudice to more clearly define the invention. Support for this amendment can be found throughout the specification as originally filed, at least, for example, at page 8, lines 12-18. No new matter enters by way of this amendment.

Withdrawn Claim Rejection

Applicants thank the Examiner for withdrawing the rejection of claims 65, 66, 69, and 70 under 35 U.S.C. § 112.

Maintained rejection under 35 U.S.C. § 112, New Matter

Claims 69 and 70 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner alleges that the instant specification is devoid of description for "wherein the rate of hypermutation in said genetically modified lymphoid cell is at least ten times higher than the mutation rate in said lymphoid cell." *See* Office action at pages 2-3.

The Examiner alleges that "[t]he cited paragraph is [] not directed to genetically modified hypermutation lymphoid cells" and that "[a] chicken lymphoid DT cell for example is capable of inducing hypermutation prior to any genetic modification." Office action at page 3. The Examiner does not provide any basis or support for these statements, and they are directly contradicted in the specification and accompanying declaration. *See*, Specification as filed at page 8, lines 10-18 (*see also* PCT publication WO2005/080552); Declaration under 37 CFR §1.132 at paragraph 10. In the absence of genetic mutation, a chicken lymphoid DT cell undergoes only gene conversion.

While Applicants disagree with the rejection, the rejected Claim 69 is amended and Claim 70 is canceled solely to facilitate prosecution. Accordingly, Applicants request withdrawal of the Examiner's rejection.

New rejection under 35 U.S.C. § 112, New Matter

Claims 29, 60, 72, and 73 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner alleges that the instant specification is devoid of description for "wherein said transgenic target nucleic acid sequence is in the absence of an adjacent donor sequence capable of serving as a gene conversion donor [for said transgenic target nucleic acid sequence.]" Office action at page 4. The Examiner alleges that none of the support cited by Applicants addresses adjacent donor sequences. Applicants disagree.

"If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met." MPEP § 2163. Applicants describe the claimed subject matter, including the aspect of hypermutation of the target nucleic acid sequence in the absence of gene conversion donors, which can also be referred to as adjacent donor sequences. Applicants even suggest a mechanism of "conversion in the presence of adjacent donor sequences and [ ] point mutation in their absence." Specification as filed at page 13, lines 23-24. The fluctuation between gene conversion in the presence of homologous adjacent sequence and hypermutation in the absence of homologous adjacent sequence is repeated throughout the specification. Specification as filed, for example, at page 8, line 33 through page 9, line 9. This reciprocal relationship was identified for the first time in the instant specification. *Id.*, *see, also*, Declaration at paragraph 4. For example, "[p]seudogenes with sequences identical to a certain region of the target gene can also be used to keep a part of the target nucleic acid stable by frequent conversions having the effect that the hypermutations persist only in the non-converting part." Specification as filed at page 9, lines 21-23.

Gene conversion will occur if there is adjacent sequence capable of homologous recombination with the nucleic acid sequence in the immunoglobulin locus. In an unmodified lymphoid cell, pseudogenes are homologous to the sequence of the immunoglobulin V gene, and gene conversion occurs. In a genetically modified lymphoid cell, in the absence of adjacent sequences capable of homologous recombination with the sequence in the immunoglobulin locus, hypermutation of the sequence in the immunoglobulin locus occurs instead of gene conversion.

Gene conversion can be prevented by either a change in the endogenous pseudogene sequences or a change in the endogenous immunoglobulin V gene sequence, or a change in both. An example of the latter case is a target nucleic acid (transgene) inserted into an immunoglobulin locus replacing the V gene. In one such situation, the transgene does not have its own gene conversion donor sequences added to the immunoglobulin locus so that the endogenous pseudogenes are no longer gene conversion donors for the transgene as they do not have sequence homology to the target nucleic acid. The specification states that "[a] 'nucleic acids [(sic)] capable of serving as a gene conversion donor' is a nucleic acid having a sequence homologous to the target nucleic acid." Specification as filed at page 11, lines 15-17. As such, nucleic acid sequences not having homology to the transgenic target nucleic acid (transgene), such as the endogenous pseudogenes in this example, are not capable of serving as gene conversion donors. Accordingly, a transgenic target nucleic acid sequence described in the specification can lack an adjacent donor sequence capable of serving as a gene conversion donor for the transgenic target nucleic acid sequence, and hypermutation occurs instead.

This method is illustrated in the specification using GFP to replace the immunoglobulin V gene in the immunoglobulin locus.

> The GFP gene inserted into the Ig light chain locus by targeted integration will be subjected to hypermutation and its activity with respect to color, intensity and half-life will evolve with time (Fig. 7B).... An Ig Vj replacement vector, pVjRepBsr, which allows to replace the Ig light chain VJ gene by any nucleic acid target is depicted in Fig. 7A. A potential target for the mutagenesis can be cloned into SpeI site... For example, the GFP gene can be inserted in to the Ig light chain locus by targeted integration using pVjRepBsr.

Specification as filed at page 19, line 30 through page 20, line 2.

The Examiner alleges that the closest subject matter to Claim 72 and 73 "refers to an embodiment, wherein 'additional nucleic acids capable of serving as gene conversion donors are inserted into the cell genome, preferably upstream of the target nucleic acid.'" Office action at page 4. The Examiner's quote refers to genetic diversification "by a combination of hypermutation and gene conversion." Specification at page 11, lines 10-11.

Depending on the amount of homology between a target nucleic acid sequence and an adjacent gene conversion donor sequence, a combination of hypermutation and gene conversion will occur. The instant specification describes that in an immunoglobulin locus containing a target nucleic acid sequence introduced into the immunoglobulin locus together with its own gene conversion donors, *i.e.*, sequences that share homology, a combination of hypermutation and gene conversion will occur. Such a method is exemplified in the specification using GFP as the sequence in the immunoglobulin locus. "If a combination of hypermutation and gene conversion is used to modify the GFP activities, variant GFP sequences which can serve as gene conversion donors for GFP are also inserted into the Ig locus (Fig. 7D)." Specification as filed at page 19, lines 27-29.

In contrast, Claim 72 recites "the absence of an adjacent donor sequence capable of serving as a gene conversion donor." In this case, only hypermutation occurs since no homologous gene conversion donor is present. For a combination of gene conversion and hypermutation as suggested by the Examiner, "additional nucleic acids capable of serving as gene conversion donors" are added to the immunoglobulin locus in addition to the transgenic target nucleic acid sequence. Both situations are described in the instant specification, for example, "the cell is constructed by replacing the endogenous V-gene or segments thereof with a transgene, by homologous recombination, or by gene targeting, such that the transgene becomes a target for the gene conversion and/or hypermutation events." Specification as filed at page 11, lines 23-26. Whether gene conversion, hypermutation, or a combination of both occur depends on the homology of the transgene and an adjacent nucleic acid sequences capable of serving as gene conversion donors.

Accordingly, a transgenic target nucleic acid sequence in the absence of an adjacent donor sequence capable of serving as a gene conversion donor for said transgenic target nucleic acid sequence is directly derivable from the specification as filed, at least, for example, from page 13, lines 21-23. As such, Applicants submit that the Examiner's rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, Written Description

Claims 29, 35, 44-56, 58-61 and 63-73 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner alleges that "[t] he specification is silent [ ] on any other genetically modified variants of lymphoid cells from any species of animals, or a DT40 or similar cell that has a hypermutation rate higher than the rate of its non-genetically modified counterpart that contains ψV donors." Office action at page 5.

"A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. *See, e.g., In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description." MPEP §2163.04.

Applicants do not understand <u>any</u> basis in any of the Restriction Requirement, four (4) Office actions on the merits, and Advisory Action issued so far by this Examiner, and therefore, submit that the written description rejection is improper. "The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. *Wertheim*, 541 F.2d at 263, 191 USPQ at 97." MPEP §2163.04. No evidence has been presented to suggest that the hypermutation of a transgenic target nucleic acid sequence does not take place where there is no gene conversion donor. There is no gene conversion donor in the absence of homology between the adjacent sequences in the immunoglobulin locus and the target nucleic acid sequence in the immunoglobulin locus.

The Examiner has invited Applicants to "explain how hypermutation is activated following ψV donor ablation, when they state that hypermutation is present in the presence of endogenous ψV genes." Office action at page 6. The explanation is that in both cases, there is no gene conversion donor in the immunoglobulin locus with homology for the target nucleic acid sequence. As such, gene conversion cannot take place. In the first situation mentioned by the Examiner, ψV donor ablation leads to the activation of hypermutation in the endogenous immunoglobulin gene. This is illustrated in Example 1 of the instant specification.

The second situation mentioned by the Examiner refers to an example where the endogenous ψV donors remain in the locus, but a transgenic target nucleic acid without homology to the endogenous ψV donors is inserted into the immunoglobulin V gene locus. As this transgenic nucleic acid sequence does not have any gene conversion donors in the locus, it is diversified by hypermutation. Again, absent any homology to the transgene, the endogenous ψV genes cannot play the role of gene conversion donors for the transgene. Therefore, in both situations, the transgenic target nucleic acid sequence has insufficient homology with the adjacent sequence in the immunoglobulin locus to undergo homologous recombination or gene conversion. While the claimed invention is not limited to any particular mechanism, the lack of homology in both situations mentioned by the Examiner prevents gene conversion so that hypermutation occurs instead.

The Examiner also inquires about Figure 7C from the instant specification. *Id.* This example demonstrates replacement of the endogenous upstream pseudo-V genes with gene conversion donors for the target nucleic acid sequence, which is GFP in this example. Examples of gene conversion donors for the GFP target sequence include pseudo-CFP, pseudo-YFP and pseudo-GFP to be transfected into the immunoglobulin locus, so that gene conversion using these donors can take place with GFP. *See*, Figure 7C. Gene conversion would not be expected to take place with endogenous pseudo-V-genes because GFP has no homology to the endogenous pseudo-V-genes to allow homologous recombination to occur. The Examiner has not shown sufficient evidence or reasoning to the contrary. Whether the endogenous pseudo-V-genes are removed does not change how the GFP gene is diversified. In fact, the pseudo-V

genes in Example 2 are removed for purely technical reasons connected with the construction of a transgenic Ig locus.

The Examiner has not provided any evidence or reasoning to suggest that hypermutation of a transgenic nucleic acid sequence in the immunoglobulin locus is affected by the presence of endogenous pseudo-V genes. The instant specification describes the opposite. For example, the situation illustrated in Figures 7A and 7B is the insertion of a GFP gene in place of the endogenous V gene where the pseudo-V genes are not deleted. *See*, Declaration at paragraph 11. Accordingly, the instant specification describes that hypermutation of a transgenic nucleic acid sequence would occur in a lymphoid cell capable of gene conversion prior to transfection where the only change was insertion of the transgenic nucleic acid sequence, such as GFP in Example 2. If this explanation is not sufficient, Applicants invite the Examiner to call or accept this invitation for another in-person interview.

The Examiner alleges that "a skilled artisan would readily recognize that any wild-type gene conversion-active cell would not be capable of having a hypermutation activity rate higher than the mutation rate of itself, absent the activation of the AID system by removal of the ψV donors." Office Action at page 7. The Examiner does not present any evidence to support this allegation beyond confusion about the instant specification. Applicants have suggested a possible mechanism for why removal of endogenous ψV donors is not required for hypermutation. The Examiner, however, has not suggested any rationale that would put the proposed mechanism or conclusions therefrom into question. Accordingly, in the absence of any reasonable basis to challenge the adequacy of the written description, the rejection is improper and should be withdrawn.

Rejections under 35 U.S.C. § 112, Enablement

Claims 29, 35, 44-56, 58-61 and 63-73 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner alleges that "the claims lack the requisite method steps" since "the genes or proteins controlling gene conversion and hypermutation must be selectively modified." Office action at page 8.

As noted by the Examiner, Applicants previously respectfully requested that the Examiner provide a basis for this rejection by directing Applicants to the evidence relied on. *Id.* at page 9. Instead of providing a basis, the Examiner recites claim language and the chicken DT40 $AID^{R}V\psi^{-}$ lymphoid cell system. That is not a basis for a proper enablement rejection.

In fact, the Examiner acknowledges that the chicken DT40 $AID^{R}V\psi^{-}$ lymphoid cell system is enabling. *Id.* The Examiner further cites the "post-filing art of Arakawa et al. … [as teaching] the stepwise removal of ψV donors not only reduces and eventually abolishes Ig gene conversion, but also activates AID-dependent Ig hypermutation in DT40 B-cell line." *Id.* at page 10. Again, there is no evidence or even rationale for a proper basis for rejection. Hypermutation following removal of endogenous ψV donors is consistent with a mechanism proposed in the instant specification. As such, this is an improper basis for rejection.

The Examiner alleges that there is an "issue [with] the ability to hypermutate a target sequence at a rate higher than the background mutation rate of the lymphoid cell." *Id.* at page 10. Even though there is still no evidence of an issue, nor suggestion why any other result would occur, *arguendo*, Applicants point to results from an experiment described in post-filing date publication Arakawa *et al.*, *Nucleic Acids Research* 36(1): e1, 2008 (previously submitted as Document BK1 on February 12, 2010). The instant specification also describes such an experiment and predicts the results. Example 2 at pages 19-20 of the instant Specification. The knock-out vector, *pHypermut1-eGFP*, was used to insert a GFP gene into the immunoglobulin light chain locus of DT40 (IgL^{eGFP1}). *See* Arakawa *et al.* at Figure 1A; *Id.* at pages 3 to 5; Declaration at paragraph 11. Hypermutation at a high rate was reported for the GFP transgene. Arakawa *et al.* at page 3, right-hang column, second section; *see, also,* Declaration at paragraph 11.

The Examiner further alleges that "the post-filing Blagodatski reference actually indicates that hypermutation of the Ig genes requires the activation of AID." Office action at page 10. Even if true, there is no evidence or even suggestion by the Examiner that AID is not constitutively active in lymphoid cells capable of gene conversion prior to transformation. *See,* Declaration at paragraph 12. The Examiner also alleges that "the post-filing Blagodatski reference actually indicates … that further Ig-related cis-acting sequences are required to

predispose neighboring transcription units to hypermutation." Office action at page 10. Solely in order to facilitate examination, Claim 60 has been canceled.

For at least the reasons noted above, a proper rejection has not been established since the Examiner has failed to establish a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure. Accordingly, Applicants respectfully request withdrawal of this rejection.

Conclusion

In view of the above, each of the presently pending claims is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejections of the claims, and to pass this application to issue. The Examiner is encouraged to contact the undersigned at (202) 942-5186 should any additional information be necessary for allowance.

Respectfully submitted,

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